REMARKS

Sua sponte amendments

Claim 1 has been amended to outline the sub-steps a)-f) of step a) to i)-vi) for clarity. Claim 8 has similarly been amended to outline the steps of the claim as a)-e) instead of 1)-5).

Claim objections

Applicant asks that objections to Claims 1, 2, and 8 with respect to their reading on non-elected inventions be held in abeyance until allowance. Applicant recognizes that Claims 1, 2, and 8 read on both elected and non-elected inventions and that claim amendments would be required if no generic claim is ultimately allowed. The word "result" in line 2 of Claim 1 has been omitted from the claim. Claim 1 identifiers a., b., and c. have been amended to a), b), and c), respectively. Claim 5 has been cancelled.

Rejections under 35 USC 112, first paragraph

Claims 1-8 and 12 have been rejected under 35 USC 112, first paragraph as failing to comply with the enablement requirement. More specifically, the Patent Office states:

> The specification discusses that the invention features a method of altering the sex ratio of transgenic offspring by expression of a toxin in spermatids containing a particular sex chromosome...However, the specification fails to provide any relevant teachings, specific guidance, or working examples with regard to generation of transgenic offspring having altered sex ratios. Furthermore, the specification fails to even describe any offspring having altered sex ratios produced by the claimed methods.

In response, Applicant notes that a working example is not a requirement for providing an enabling disclosure. Applicant's disclosure provides in detail a description for how to make and how to use the claimed invention, absent a working example. Applicant's claims for purposes of examination have been limited to methods comprising pronuclear injection (see *Claim objections*, above). One skilled in the art would predict with a high degree of certainty that an expressible transgene of Claims 1-8 and 12 could be made via pronuclear injection. While not all injections would yield germline transformations, a significant percentage of injected non-human animals would be expected to yield the desired offspring. With certainty, one skilled in

the art would be able to make the transgenic constructs of Claims 1-8 and 12 using sequence information and DNA constructs available in the art. Applicant's description of said sequences provides sufficient guidance for the ordinary molecular biologist to make and use the invention of said claims. While one cannot predict with certainty the chromosomal location of insertion of any particular transgenic element introduced via pronuclear injection, and hence that the element would be expressible, one skilled in the art would expect that a particular fraction of constructs would integrate into a region suitable for gene expression of said construct. Obtaining germline transformants thus may require multiple rounds of injections and subsequent characterization of the germline transformants. Applicant does not, however, characterize the generation and subsequent characterization of multiple lines of transgenic animals as "undue experimentation." It is within the knowledge of one skilled in the art to provide insulator sequences flanking the transgenic construct, to shield it from local chromatin structure which may negatively affect its expression. It is with a high degree of certainty that, given the desired expression of the transgene within the animal, one skilled in the art would expect the claimed method to yield the desired sex ratio in germline transformants, wherein the sex of said animal is determined by the sex chromosome of the sperm. The Patent Office further states:

> ...As the specification fails to provide any relevant teachings or guidance with regard to the production of such transgenic, as embraced by the claims, one of skill in the art would not be able to rely on the state of the transgenic art for an attempt to produce transgenic animals that comprise and express any heterologous nucleotide sequence encoding a toxin...the level of expression of a transgene as well as the resulting phenotype of the transgenic animal are directly dependent on the specific transgene construct...

In response, Applicant has amended Claim 1 to specify that the nucleotide sequence encoding the toxin is the thymidine kinase (tk) gene, or its mutated or truncated forms. Claim 1 has been further amended to specify that the transgene promoter is specifically Herpes Simplex Virus promoter (HSV), or its mutated or truncated forms. As stated in the specification, HSV-tk contains a spermatogenesis-specific promoter, and that the promoter is able to drive expression at sufficient levels to disrupt spermatogenesis (for references see page 4 of the specification). As stated above, while one cannot predict with certainty the chromosomal location of insertion of any particular transgenic element introduced via pronuclear injection, and hence that the element would be expressible, one skilled in the art would expect that a particular fraction of

constructs would integrate into a region suitable for gene expression of said construct. Obtaining desired germline transformants thus may require multiple rounds of injections and subsequent characterization of the germline transformants. Applicant does not, however, characterize the generation and subsequent characterization of multiple lines of transgenic animals as "undue experimentation." As stated above, it is within the knowledge of one skilled in the art to provide insulator sequences flanking the transgenic construct, to shield it from local chromatin structure which may interfere with its expression.

The Patent Office further states:

> Strojek and Wagner (*Genetic Engineering*, 1988) pointed out that a high degree of expression of a transgene in a mouse is often not predictive of high expression in other species, including pigs and rabbits, because, for example, the cis acting elements may interact with different transacting factors in these other species...Given such species differences in the expression of a transgene, particularly when taken with the lack of guidance in the specification for the production of transgenic animals comprising any heterologous nucleotide sequence encoding a toxin, it would have required undue experimentation to predict the results achieved in any one host animal comprising and expressing any heterologous sequence encoding a toxin, the levels of the expression product, the consequences of that production, and therefore, the resulting phenotype of elimination of a spermatid comprising a particular sex chromosome.

Applicant has amended Claims 1-8 and 12 to limit the scope of the invention to the specific toxin described in the specification, the Herpes simplex virus thymidine kinase (HSV-TK), and variants thereof, which include mutated or truncated forms. While the levels of expression of such a transgene may vary from species to species, one of skilled in the art would expect with almost certainty that a transgene of the present invention would be effective in altering the sex ratio of transgenic offspring. While a transgene of the present claims may not be effective in **preventing** offspring of one sex versus the other in all species, one skilled in the art would predict with a high degree of certainty that for any animal specie wherein sex is determined by the sex chromosome of the sperm, a transgene of the claimed invention would be effective in, at the very least, **altering** the sex ratio of males to females in offspring of a founder animal. If levels of the transgene are lower in one specie relative to another, one skilled in the art would predict that the ratio of males:females would, at the very least, be biased in the desired direction. In cases where the methods of the present invention may work less well in one

specie relative to another, possession of the desired germline transformants may merely require more rounds of injections and subsequent characterizations of the germline transformants. Applicant does not, however, characterize the generation and subsequent characterization of more lines of transgenic animals in one specie over another as "undue experimentation."

The Patent Office further states:

> The specification has failed to provide guidance correlating to targeting of a transgene to specific loci of the sex chromosomes using methodology that relies on random transgene integration. It is unpredictable if random integration of a transgene would enable targeting to specific loci of sex chromosomes. Given the lack of guidance with respect to targeting of specific sex chromosome loci by random integration provided by the instant specification it would have required undue experimentation to practice the claimed invention.

While one cannot predict with certainty the chromosomal location of insertion of any particular transgenic element introduced via pronuclear injection, one skilled in the art would expect that a particular fraction of constructs would integrate into an expressible region on the desired sex chromosome. As stated in the specification (page 6, lines 3-4), subsequent to introduction of the trangene into the genome of an animal, one skilled in the art could use FISH to screen transgenic founders to identify with absolute certainty those individuals bearing the transgene on the desired sex chromosome. Obtaining the desired germline transformants thus may require multiple rounds of injections and subsequent characterization of the founders via FISH. One skilled in the art would predict that insertion of said transgene onto the desired sex chromosome is with almost certainty obtainable, provided that enough injections and subsequent characterizations have been done. Applicant does not, however, characterize the generation and subsequent characterization of multiple lines of transgenic animals as "undue experimentation."

The Patent Office further states:

> When the loxP sites are not included in the claimed invention, the required step of introducing Cre recombinase activity into an animal of the invention appears to have (sic) apparent function or purpose.

In response, Applicant has amended Claim 1 step c) to recite the limitation "wherein the transgene includes the optional loxP site-flanked intervening DNA sequence of step a) iii)" to indicate that the transgenic males be mated to females bearing Cre recombinase only when their transgenes harbor the LoxP sites. As a result, Applicant has added new step d) to Claim 1, "identifying at least one transgenic animal with desirable reproduction feature, specifically, alteration of offspring's sex ratio, and thereby altering the offspring sex ratio of said animal."

The Patent Office further states:

> ...when the LoxP sites are not part of the invention, it appears that toxin expression would be ubiquitous and could result in embryonic lethality of founder animals or in elimination of sperm production of founder animals. Accordingly, the claims do not appear enabled for production of transgenic animals having altered sex ratios when loxP sites are not part of the invention.

In response, Applicant notes that the toxin is expressed solely under the control of a spermatogenesis specific cryptic promoter, as stated in the specification on page 4, lines 16-21. Therefore the toxin is only expressed in sperm cells during spermatogenesis. The toxin resultingly could not detrimentally affect the health of the animal bearing the trangene. It is not an essential element of the invention that the transgenic animals be maintained as a stable "stock." Further, the optional diptheria toxin gene would only be built into the transgene wherein the transgene is to be introduced via homologous recombination, and as such would be recombined away upon introduction into the animal.

Claims 1-8 and 12 have been rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement. More specifically, the Patent Office states:

> Based upon the prior art there is expected to be variation among the species of cDNA, which encode toxins, because the sequence of toxin cDNAs would be expected to vary. There is no evidence on the record that the HSV-tk cDNA has a known structural relationship to any other toxin encoding cDNA sequences, which when expressed interfere with sperm's ability to fertilize and diffuse in a limited manner among the inter connected spermatids; the specification discloses only an HSV-tk cDNA...Consequently since Applicant was in possession of only the HSV-tk cDNA and since the art recognized variation among the species of the genus of cDNAs that encode toxins, the HSV-tk cDNA was not representative of the claimed genus.

This rejection has been obviated by amendment. More specifically, Claim 1, step a) ii) has been amended to recite the limitation, "wherein the toxin is selected from the group consisting of thymidine kinase (tk), its mutated, and truncated forms."

Rejections under 35 USC 112, second paragraph

Claims 1-8 and 12 have been rejected under 35 USC 112, second paragraph as being indefinite. The "such as" language has been omitted from Claim 1, and the phrase following this language has been incorporated into new Claim 13. The parenthetical material of Claim 1 has been deleted.

Claim 1 has been rejected because it is incomplete as written. More specifically, the Patent Office states that the steps relating to the creation of transgenic animals are not included in the claim. Furthermore, the Patent Office states that the steps of the method do not relate to the goal of the preamble in a positive process. In response, Applicant has amended the preamble to "a method for altering the offspring sex ratio of an animal..." The preamble as amended relates to the goal of the claim in a positive process, and the steps of this method are outlined in the claim.

The Patent Office states that there is insufficient antecedent basis for "toxin gene" and "toxin transgene" in Claim 1. Claim 1 is also considered indefinite because step b) is directed to a DNA sequence encoding a gene. Claim 1 has been amended to obviate this rejection. More specifically, "toxin gene" has been changed to recite, "sequences encoding the...toxin," and "toxin transgene" has been amended to "toxin." Applicant submits that there is sufficient antecedent basis for the amended language and that the amendments obviate the rejections.

Claim 1 is considered indefinite because it is unclear whether said claim is directed to a method for producing transgenic animals whose somatic or germ cells, or both, contain transgenes. The "somatic/germ cells" language has been omitted from the claim by amendment.

Claim 1 is considered indefinite because it incorrectly recites the phrase "sperm's ability to undergo fertilization." This phrase of Claim 1 has been amended to "sperm's ability to fertilize an oocyte," thereby obviating this rejection.

Claim 1 is considered indefinite because it is unclear what is meant by "functional in a post-meiotic spermatogenesis-specific way." In response to this rejection, the phrase has been

amended to define the regulatory sequence as a post-meiotic spermatogenesis-specific regulatory sequence. The phrase as amended is now definite.

Claim 2 has been rejected as being indefinite since the claim embraces animals that are unisexual flower plants. Since methods for producing transgenic unisexual flower plants were non-elected for prosecution in this case, the claim has been amended to omit the non-elected invention from the claim. The amendment obviates the rejection.

Claim 3 has been considered indefinite. The range of the claim has been considered unclear. The Patent Office further rejects the claim because the phrases "its mutated or truncated genes," "toxic genes with characters," its expression can interfering," mRNA/protein products act in a no-random diffusion," mRNA/protein products," and sperm's ability to undergo fertilization" are not clear. Claim 3 has been cancelled, thereby obviating rejections of this claim.

Claim 4 has been rejected for indefiniteness because there exists no antecedent basis for the phrase "the offspring's desirable sex percentage of said transgenic animals." This claim has also been rejected because this phrase is unclear in meaning. In response, Applicant has amended the phrase to "alteration of offspring's sex ratio," for which there is sufficient antecedent basis.

Claim 5 has been rejected because the limitation "said post-meiotic spermatogenesis-specific promoter" lacks antecedent basis. Claim 5 has been cancelled, thereby obviating this rejection.

Claim 6 has been rejected because the phrase "said the DNA sequence" is unclear and further because the phrase "said the DNA sequence for X-chromosome specific targeting" lacks antecedent basis. In response, Claim 6 has been amended to provide clarity and antecedent basis.

Claim 7 has been rejected because the phrase "said the DNA sequence for Y-chromosome specific targeting" has insufficient antecedent basis. In response, Claim 7 has been amended to provide clarity and antecedent basis.

Claim 8 has been rejected because the phrases "the post-meiotic spermatogenesis-specific promoter," "the transgene that disrupts sperm's function," and "the toxin gene" have insufficient antecedent basis. Furthermore, "such as" limitations, parenthetical expressions, and the phrase "a DNA sequence encoding a toxic gene" have been considered unclear. In

response, Claim 8 has been amended to provide sufficient antecedent basis for the phrases. The "such as" limitations and parentheses have been deleted.

Rejections Under 35 USC 102

Claims 1-5 and 8 have been rejected under 35 USC 102(b) as being anticipated by Silversides et al (US Pat. No. 5, 596,089). More specifically, the Patent Office states:

> Silversides et al. teach methods of altering the sex ratios of transgenic offspring comprising: 1) preparing a transgene comprising a spermatogenesis-specific promoter (the SRY promoter) operably linked to a nucleotide sequence encoding a toxin (Diptheria A chain toxin), wherein loxP sequences flanking an intervening sequence are positioned between the promoter and the toxin gene as they have been inserted into the transgene immediately downstream of the transcriptional start site of the Diptheria A toxin gene; 2) creating transgenic animals comprising the above described transgene...

Applicant respectfully submits that the Claims 1-5 and 8, as amended, are free of the cited art. Silversides et al. does not anticipate all of the instant claim limitations. Silversides et al. does not teach a method of altering the sex ratios of transgenic offspring comprising preparing a transgene comprising a post-meiotic spermatogenesis-specific expression regulatory sequence wherein the regulatory sequence is selected from the group consisting of a Herpes Simplex Virus promoter (HSV) promoter, its mutated, and truncated forms, as recited in Applicant's Claim 1. Claims 2-5 are dependent on Claim 1, and thus also recite this distinguishing limitation. Silversides et al. does not anticipate Claim 8 since the reference does not teach the limitation of Claim 8 as amended, "preparing a transgene which comprises in operable association of at least one expression regulatory sequence which expresses in early stage embryos but not during spermatogenesis for XY organisms or oogenesis for ZW organisms." Applicant respectfully submits that the rejected claims are free of the cited art.

Claims 1-5 have been rejected under 35 USC 102(b) as being anticipated by Eisel et al. More specifically, the Patent Office states:

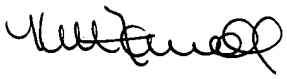
> Eisel et al. teach the creation of transgenic mice whose genomes comprise a transgene comprising a nucleotide sequence encoding tetanus toxin operably linked to a promoter functional in a post-meiotic spermatogenesis-specific way as expression was achieved in the testes.

Applicant respectfully submits that Claims 1-5 as amended are free of the cited art. Eisel et al. does not teach a method of altering the sex ratios of transgenic offspring comprising preparing a transgene comprising a post-meiotic spermatogenesis-specific expression regulatory sequence wherein the regulatory sequence is selected from the group consisting of a Herpes Simplex Virus promoter (HSV) promoter, its mutated, and truncated forms, as recited in Applicant's Claim 1. Claims 2-5 are dependent on Claim 1, and thus also recite this distinguishing limitation.

Summary

In light of the above amendment, consideration of the subject patent application is respectfully requested. Any deficiency or overpayment should be charged or credited to Deposit Account No. 500282.

Respectfully submitted,



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